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FACTORS AFFECTING THE RECOVERY OF HYDROCYANIC ACID FROM FUMIGATED CITRUS TISSUES[1,2]

E. T. BARTHOLOMEW,[3] WALTON B. SINCLAIR,[4] AND BYRON E. JANES[5]

UNDER CERTAIN conditions of fumigation for scale insects of citrus trees, hydrocyanic acid (HCN) is known to cause injury to the foliage and fruit. The physiological reactions involved in the injury have received but slight quantitative investigation. Undoubtedly the reasons for this have been the difficulties encountered in distilling HCN from materials containing volatile substances which are reactive with HCN. The lack of a method with sufficient accuracy to recover relatively small amounts of HCN from the tissues may also have been an important factor.

The results reported in this paper are concerned only with the factors affecting the distillation and recovery of HCN from solutions in the absence of tissues and also in the presence of citrus foliage and fruits. Earlier attempts to recover HCN from fumigated citrus tissues by aspiration, partial vacuum, immersing and shaking the tissues in an alkaline solution, or by a combination of these processes have given unsatisfactory results. All statements in this paper concerning the recovery of HCN from citrus tissues refer to HCN added by fumigation or by other methods. Repeated tests by Bartholomew and Raby (*3*) and in the present investigation have shown that citrus tissues do not contain autogenous HCN.

An investigation is being made of the physiological effects of HCN in citrus tissues.

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[2] Paper No. 390, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

[3] Plant Physiologist in the Experiment Station.

[4] Junior Plant Physiologist in the Experiment Station.

[5] Laboratory Assistant in Plant Physiology in the Experiment Station.

THE QUANTITATIVE DETERMINATION OF HCN IN THE ABSENCE OF CITRUS TISSUES

A modified Liebig silver-nitrate–volumetric method was employed for the determination of HCN, the end points of titration being determined by the use of a photoelectric turbidimeter. The operations were conducted according to the recommendations made by Bartholomew and Raby (*2,3*). Preliminary experiments, however, demonstrated that a new method of recovery of HCN must be developed or an old one be adapted to the material to be investigated.

Considerable difficulty was experienced in weighing accurately such small amounts of liquid HCN as were being used. The first method was to seal 30 to 40 mg of liquid HCN in a weighed 5-ml glass ampoule. When the ampoule was broken in a liter of 0.1 *N* NaOH and 150-ml aliquots titrated with standard $AgNO_3$, the total amount of HCN determined was 104 to 106 per cent of the quantity originally weighed. The possible sources for this error were in the method of titration and in the method of weighing the liquid HCN. The results recorded in table 1, which show the recovery of HCN from NaCN, demonstrated that the titration method was accurate.

TABLE 1

AMOUNTS OF HCN RECOVERED FROM KNOWN QUANTITIES OF NaCN DISSOLVED IN 0.1 *N* NaOH SOLUTIONS

Experiment No.	Purity of NaCN per cent	NaCN added, mg	HCN equivalent, mg	HCN recovered	
				Amount, mg	Per cent
130	95.5	58.9	31.0	30.5	98.4
131	96.5	58.3	31.0	30.9	99.7
132	96.5	60.0	31.8	31.8	100.0
133	96.5	30.0	15.9	16.0	100.6

This left as the only other source of error, the inaccurate method of weighing: if a small amount of liquid HCN (30–40 mg) is placed in a weighed 5-ml ampoule and a second weighing is made, the difference between the two weights does not show the exact amount of HCN in the ampoule because a certain amount of the air is replaced by HCN vapor, which is lighter.[6] The actual amount of air displacement in the larger

[6] For example, at 23° C and 750-mm pressure and with a difference of 31 mg between the first and second weighings, the ampoule (5-ml capacity) would contain an excess of 19 per cent of HCN if all the air (5.9 mg) were displaced. Liquid HCN has a low boiling point—26°—and an exceedingly high vapor pressure at ordinary laboratory temperatures—654.4 mm at 22° (*9*).

containers could not be governed or determined; therefore the amount of HCN existing in the vapor phase was reduced to a minimum by using ampoules with a volume just equivalent to, or only slightly greater than, the amount of liquid HCN desired for a given test. This eliminated the difficulty.

The quantities of HCN used in the experiments were small, never over 100 mg, the average amount being about 39 mg. The laboratory fumigation chamber has a capacity of 5 liters, and the total volume of the solutions used for distillation was 3 liters. As shown by these volumes, the actual concentrations of HCN used in the experiments were very low. Before the experimental methods could be applied to citrus tissues, the conditions for the distillation and recovery of pure samples of HCN in such concentrations had to be standardized.

TABLE 2

AMOUNTS OF HCN RECOVERED AFTER VARIOUS TREATMENTS

Treatment (liquid HCN used)	Experiment No.	HCN added, mg	HCN recovered	
			Amount, mg	Per cent
A, In empty fumigation flask 45 min., then acidulated H_2O and $CdSO_4$ added	163	41.6	41.2	99.0
	165	23.5	23.5	100.0
	166	33.9	33.9	100.0
	167	11.0	11.0	100.0
B, In 0.1*N* NaOH without distillation	146	897.9	901.0	100.3
	147	16.7	17.0	101.8
	148	22.0	21.8	99.1
	149	44.2	44.4	100.5
	150	28.6	29.3	102.4
	153	53.2	53.5	100.6
C, In 0.1*N* NaOH with double distillation, acidulated H_2O and $CdSO_4$ added	218	33.7	33.5	99.4
	219	26.1	25.8	98.9

Having solved the difficulties previously encountered in weighing known amounts of HCN, the first step in standardizing the methods for its recovery was to break the ampoule of liquid HCN in the empty fumigation flask and allow it to remain for 45 minutes. Thirty grams of $CdSO_4$ were added and enough H_2SO_4 in each case to make the solution slightly acid—0.15 or 0.30 ml beyond neutrality (p. 477), according to the nature of the solution to be distilled. (The reason for adding the $CdSO_4$ will be explained in the following section.) The results of these tests demonstrated that no losses occurred through the apparatus connections. The tabulated results are shown as treatment A in table 2.

The second step was to break ampoules containing known amounts of

liquid HCN in the bottom of a tall cylinder containing about 900 ml of 0.1 *N* NaOH. The solution was then poured into a liter volumetric flask and diluted to volume. The solution was not distilled; therefore no acid or $CdSO_4$ was added. Aliquot samples of 150 ml each were titrated with standard $AgNO_3$. The amounts recovered were in agreement with the available amount in each sample, as shown for treatment B in table 2.

The third step was to attempt to recover the HCN from 0.1 *N* NaOH by double distillation, after having added the usual amounts of H_2SO_4 and $CdSO_4$. The first distillates were redistilled because, as explained elsewhere in this paper, the first distillate from fumigated leaves contained a volatile substance which interfered with the titration of the HCN. The data (treatment C in table 2) show that the double distillation process resulted in only a very slightly lower percentage recovery of HCN than treatment B.

THE RECOVERY OF HCN FROM CITRUS-LEAF DISTILLATES

General Methods with Leaves.—Mature or young citrus leaves were picked from the trees, placed in a container with a tight cover, and immediately brought to the laboratory and thoroughly mixed. About 15 to 20 minutes elapsed between picking the leaves and placing them in the fumigation flask. The moisture in each lot of leaves was determined on a 100-gram sample by heating the leaves at 100° C for 1 hour and then to constant weight at 70°. The loss in weight was recorded as the percentage of moisture.

Unless otherwise stated, 200 grams of mature leaves were used for each fumigation experiment. The 200-gram sample was fumigated in a 5-liter Pyrex flask and the length of the fumigation period was always 45 minutes unless otherwise stated. Pyrex flasks were used in place of ordinary soda flasks because sodium cyanide would have been formed on the walls of the latter (*9*). A small ampoule containing a known amount of HCN was broken in the flask containing the leaves. The flask was closed with a rubber stopper containing a large glass tube and a separatory funnel, both of which contained stopcocks so that an airtight seal could be made and no appreciable loss of HCN would occur. Tin foil was firmly cemented to the lower surface and sides of the stopper so that no reaction could take place between the rubber and the HCN (*5*). The flask was kept in an inverted position and shaken several times during the fumigation period.

At the end of the fumigation period, the flask was connected to a condenser by means of the large glass tube. The end of the condenser extended to the bottom of a 1-liter volumetric flask containing 100 ml of

N NaOH. The large stopcock in the tube leading to the condenser was opened, and 3 liters of acidulated distilled water was added through the separatory funnel. Enough material was distilled over to make 1 liter in the receiving flask; this brought the distillate to approximately 0.1 N with respect to NaOH. After the distillate had been filtered, 2 liters of acidulated distilled water and 30 grams of $CdSO_4$ were added, and another liter distilled and caught as before. Aliquots of 150 ml each of the second distillate were titrated to turbidity with standard $AgNO_3$. The concentration of the $AgNO_3$ was usually 0.020 N, but ranged from 0.019 to 0.022 N.

The amount of acid placed in the distillation flask depended on whether it was added directly to the fumigated tissues or to the alkaline distillate. In the earlier tests, 0.2666 N (2 per cent) tartaric acid or 0.0036 N H_2SO_4 (0.30 ml of concentrated H_2SO_4 in 3 liters of solution) beyond the calculated neutrality (of solutions) was used. Before the investigation had progressed very far, citrus tissues were found to be sufficiently acid in reaction to make possible the recovery of the HCN during the distillation process. Therefore during the later tests on the leaves and on all of the fruits, only 0.0018 N H_2SO_4 (0.15 ml in 3 liters of solution) was added. This precaution was taken to make sure that the medium would be acid in all cases.

Interference of Hydrogen Sulfide.—Difficulties were encountered at once when attempts were made to determine HCN in the distillate from fumigated leaves. The first experiments showed that the distillate contained volatile substances which were carried over with the steam and which produced a darkening of the distillate on the addition of the first increment of $AgNO_3$. Continued darkening of the solutions with further additions of $AgNO_3$ made it obvious that HCN could not be determined in the presence of such impurities. Therefore the immediate problem was to free the distillate of the volatile substances which reacted with $AgNO_3$.

There are from 2 to 4 ml of volatile oils in a liter of distillate from a 200-gram sample of leaves. The removal of most of the oil by filtration did not eliminate the titration difficulties. Quantitative tests showed that the distillate was free of organic sulfur and volatile nitrogenous compounds. The solution, however, had a slight odor of H_2S, the actual presence of which was confirmed by testing with lead acetate.

A quantitative determination of the H_2S was made by the $CdSO_4$ method, as described by Scott (*8*). A 200-gram sample of leaves was distilled and four successive portions of 200 ml each were caught in 50 ml of a 6 per cent solution of $CdSO_4$. Separate similar distillations were made with the whole fruit, peel, and pulp. As shown in table 3, the total amount

of H_2S which occurred in the distillate from the leaves or fruit was relatively small. However, when an aliquot portion was titrated with $AgNO_3$, the quantity of Ag_2S formed was sufficient to discolor the solution to such an extent that a false end point was obtained.

The problem at hand was to convert the volatile H_2S to a compound which was neither volatile with steam nor reactive with HCN. In an effort to do this, $CdSO_4$ was added just before distillation began. The H_2S reacted with the $CdSO_4$ to form CdS, which is nonvolatile and which remained in the flask during distillation. To convert all the H_2S to CdS,

TABLE 3

AMOUNTS OF VOLATILE H_2S OBTAINED FROM THE DISTILLATES OF CITRUS LEAVES AND GREEN FRUITS

Fraction (distillate) No., 200 ml each	H_2S from 200-gm samples of leaves, mg				H_2S from 15 green Valencia oranges, mg		
	Sample I	Sample II	Sample III	Sample IV	Whole fruit	Peel	Pulp
1	2.28	2.38	2.20	2.29	3.40	1.32	1.41
2	0.73	0.82	0.64	0.64	0.75	0.99	0.58
3	0.55	0.55	0.46	0.55	0.66	0.66	0.58
4	0.46	....	0.55	0.46	0.75	0.50	0.66
Total	*4.02*	*3.75*	*3.85*	*3.94*	*5.56*	*3.47*	*3.23*

however, the solution would have had to cover the leaves entirely during distillation. This was impossible because the specific gravity of the fresh leaves was less than that of the solution; hence, the $CdSO_4$, instead of being placed in the flask with the leaves, was placed in the leaf distillate, and this solution was redistilled. The double distillation process freed the solution of all H_2S.

Before applying this procedure to leaves, it was necessary to determine experimentally what effect, if any, $CdSO_4$ would have on the recovery of known amounts of HCN. This was done by breaking ampoules containing known amounts of HCN, in 0.1 *N* NaOH. This solution was then poured into the distilling flask and diluted to 3 liters. In some of the experiments H_2SO_4, and in others tartaric acid, was used. Each distillation was made in the presence of 30 grams of $CdSO_4$. The results given in table 4 show that the presence of the $CdSO_4$ did not materially reduce the percentage of recovery of HCN, and that the recoveries were the same with H_2SO_4 and with tartaric acid.

Penetration of HCN into Leaf Tissues.—A series of experiments was carried out to determine the amount of HCN that actually penetrated the leaves during fumigation. At the end of the fumigation period, the

leaves were washed in the 5-liter fumigation flask with 2 liters of 0.1 *N* NaOH by shaking thoroughly so as to get the alkaline solution to contact the walls of the flask and the surface of the leaves. The leaves were then washed four times with distilled water, 2 liters each time, and finally with 2 liters of acidulated water (containing 0.15 ml of concentrated H_2SO_4). The acid solution was poured from the leaves, then 3 liters of the acidulated water was added to the leaves in the flask and distillation conducted in the usual manner.

TABLE 4

AMOUNTS OF HCN RECOVERED AFTER DISTILLATION FROM SOLUTIONS ACIDIFIED WITH H_2SO_4 OR TARTARIC ACID

(Each solution contained 30 grams $CdSO_4$)

Acid used	Experiment No.	HCN added, mg	HCN recovered	
			Amount, mg	Per cent
H_2SO_4	151	26.1	26.2	100.4
	152	38.9	39.0	100.3
	155	34.3	34.5	100.6
	154	29.6	30.1	101.7
	161	28.8	28.7	99.7
	Average	....	....	100.5
Tartaric acid	156	53.4	53.6	100.4
	157	18.4	18.6	101.1
	158	59.9	60.0	100.2
	160	32.3	32.5	100.6
	Average	....	....	100.6

As may be seen in table 7 (p. 481) an average recovery of 66.3 per cent of the HCN added was obtained from the different lots of leaves which had received such a washing treatment in the interval between fumigation and distillation.

Retention and Fixing of HCN by the Tissues.—Another experiment was made to determine how much available HCN had remained in the leaves at given intervals after fumigation. The leaves were treated and recoveries made according to the procedure already described. At the end of the 45-minute fumigation period, the leaves were removed from the chamber, spread on ¼-inch-mesh wire screen and exposed to laboratory air for 1 minute, and for periods of 2, 4, 8, and 15 hours before they were distilled for the determination of HCN.

The results of these experiments are shown in table 5. A measurable amount of HCN remained in the leaves even after an exposure of 15 hours to the laboratory air. The HCN that could not be recovered was apparently fixed in the vapor phase or by some nonvolatile substance in the leaf tissues either before or during distillation.

In order to determine whether the reactions occurred entirely or at least largely in the vapor phases, known amounts of HCN were added to distillates from unfumigated leaves and distilled at once in the usual manner. The results of these tests, given in table 6, show an average recovery of 99.0 per cent of the HCN.

TABLE 5

AMOUNTS OF HCN RECOVERED FROM FIVE 200-GRAM LOTS OF CITRUS LEAVES EXPOSED TO LABORATORY AIR FOR DIFFERENT LENGTHS OF TIME AFTER FUMIGATION

Time aerated	Experiment No.	Moisture in leaves, per cent	HCN added, mg	HCN recovered	
				Amount, mg	Per cent
1 minute	237	56.2	48.2	29.1	60.4
	238	60.1	34.7	23.0	66.3
	241	57.0	38.7	28.3	73.1
	242	55.7	49.6	33.5	67.5
	Average	....	....	....	66.8
2 hours	222	59.5	47.3	17.5	37.0
	223	58.3	33.0	13.1	39.7
	225	59.0	44.0	16.8	38.2
	227	56.6	37.2	14.6	39.2
	Average	....	....	....	38.5
4 hours	251	54.8	28.4	7.8	27.5
	252	54.8	28.9	8.6	29.8
	264	59.1	31.2	8.3	26.6
	265	59.1	27.8	7.1	25.5
	Average	....	....	....	27.4
8 hours	266	58.0	40.2	6.3	15.7
	267	58.0	34.2	4.6	13.5
	268	59.0	32.0	4.4	13.8
	269	59.0	43.0	6.3	14.7
	Average	....	....	....	14.4
15 hours	230	58.5	37.2	7.6	20.4
	231	57.9	28.1	4.7	16.7
	239	57.3	31.2	4.2	13.5
	240	60.4	44.0	4.1	9.3
	Average	....	....	....	15.0

The next point was to determine whether additional HCN would react and combine with nonvolatile substances in the leaf tissues or with substances that were not volatile enough to be carried over during the first distillation. (Previous tests had shown that additional amounts of volatile substances were driven over when leaf tissues were distilled a second time.) For this purpose, known amounts of HCN were added to different 200-gram lots of unfumigated leaves and distilled at once. The results of these tests, also given in table 6, show an average recovery of only 95.8 per cent of the HCN.

TABLE 6

RECOVERY OF HCN THAT HAD BEEN ADDED DIRECTLY TO THE DISTILLATES FROM CITRUS LEAVES AND THEN DISTILLED OR THAT HAD BEEN DISTILLED IN THE PRESENCE OF MATURE CITRUS LEAVES

Treatment	Experiment No.	Moisture of leaves, per cent	HCN added, mg	HCN recovered	
				Amount, mg	Per cent
HCN added to leaf distillate	255	58.5	41.7	40.9	98.1
	256	57.5	35.1	34.7	98.9
	257	57.5	53.6	53.1	99.1
	258	57.5	25.7	25.7	100.0
	Average	....	....	...	99.0
HCN distilled in presence of leaves	259	57.6	45.0	43.8	97.3
	260	57.6	34.8	33.0	94.8
	261	59.5	41.2	39.0	94.7
	262	59.5	19.5	18.8	96.4
	263	57.0	25.8	24.7	95.7
	Average	....	....	....	95.8

Tests (see table 2) had shown that the HCN had not escaped from the flask; therefore it appeared that either the tissues were not finely enough divided to permit all of the HCN to escape during the process of distillation, or a certain amount of the HCN was chemically bound or changed within the tissues during the periods of fumigation and distillation. An attempt to solve this problem was made by grinding 200-gram lots of fresh leaves to pass a 2-mm screen in a Wiley mill and by exposing the lots of ground tissues to known quantities of HCN in the distillation flask

TABLE 7

AMOUNTS OF HCN RECOVERED FROM GROUND AND FUMIGATED CITRUS LEAF TISSUE AND FROM CITRUS LEAVES THAT HAD BEEN WASHED TO REMOVE HCN ADHERING TO THEIR SURFACES

Material used	Experiment No.	Moisture in leaves, per cent	HCN added, mg	HCN recovered	
				Amount, mg	Per cent
Washed leaves	232	59.5	38.7	26.4	68.2
	233	59.5	43.0	26.4	61.4
	235	59.5	28.8	20.6	71.5
	236	59.4	46.8	29.9	63.9
	Average	....	....	....	66.3
Ground leaf tissue	211	59.7	0.0	0.0	0.0
	207	60.0	42.6	33.7	79.1
	208	61.5	23.8	19.1	80.2
	209	57.6	47.5	36.8	77.5
	210	60.5	27.8	23.1	83.1
	Average	....	....	....	80.0

for a period of 45 minutes before distillation was begun. In these tests, the average recovery of HCN was only 80.0 per cent, as shown in the second group of data in table 7.

Several series of experiments were carried out to determine the effects of different experimental treatments on the amounts of HCN that could be recovered from leaves. The treatments used are shown in table 8. The double-distillation process was used in all of the tests, and either gas or

TABLE 8

AMOUNTS OF HCN RECOVERED FROM 200-GRAM LOTS OF CITRUS LEAVES FUMIGATED AND DISTILLED UNDER DIFFERENT EXPERIMENTAL CONDITIONS

Treatment*	Experiment No.	Moisture in leaves, per cent	HCN added, mg	HCN recovered		HCN not recovered by distillation	
				Amount, mg	Per cent	Amount, mg	Per cent
Series A, 100 grams of leaves, fumigated 45 minutes							
Solutions acidified with H_2SO_4	180	54.3	51.8	47.1	90.9	4.7	9.1
	181	52.5	31.7	28.2	89.0	3.5	11.0
	182	53.5	29.6	26.5	89.5	3.1	10.5
	183	52.2	32.9	29.6	90.0	3.3	10.0
	Average	....	....	....	89.9	....	10.1
Solutions acidified with tartaric acid	184	55.3	39.8	35.1	88.2	4.7	11.8
	185	55.5	41.9	37.2	88.8	4.7	11.2
	186	54.8	47.9	41.0	85.6	6.9	14.4
	187	56.0	48.3	43.4	89.9	4.9	10.1
	188	53.8	13.4	10.9	81.3	2.5	18.7
	Average	....	....	....	86.8	....	13.2
Series B, 200 grams of leaves, fumigated 90 minutes							
Solutions acidified with H_2SO_4	195	57.8	32.8	26.5	80.8	6.3	19.2
	196	58.0	34.8	29.2	83.9	5.6	16.1
	197	59.0	47.5	39.9	84.0	7.6	16.0
	198	60.0	38.4	32.6	84.9	5.8	15.1
	Average	....	....	....	83.4	....	16.6
Solutions acidified with tartaric acid	189	56.2	28.7	20.4	71.1	8.3	28.9
	190	56.7	31.9	25.1	78.7	6.8	21.3
	191	55.7	42.7	33.5	78.5	9.2	21.5
	192	55.0	48.9	39.1	80.0	9.8	20.0
	193	53.9	25.5	17.7	69.4	7.8	30.6
	Average	....	....	....	75.5	....	24.5
No acid in first distillation, H_2SO_4 used in second	202	61.5	23.3	18.8	80.7	4.5	19.3
	203	60.7	41.4	36.0	87.0	5.4	13.0
	204	55.1	37.8	33.3	88.1	4.5	11.9
	205	58.3	23.5	19.1	81.3	4.4	18.7
	Average	....	....	....	84.3	....	15.7

* All distillations were made by using direct gas or electric heat, except as indicated for th second group of tests in series C.

TABLE 8 (*Continued*)

Treatment*	Experiment No.	Moisture in leaves, per cent	HCN added, mg	HCN recovered		HCN not recovered by distillation	
				Amount, mg	Per cent	Amount, mg	Per cent
Series C, 200 grams of leaves, fumigated 45 minutes							
No acid in first distillation, H_2SO_4 used in second...	194	54.8	40.7	33.2	81.6	7.5	18.4
	199	59.7	33.1	29.0	87.6	4.1	12.4
	200	56.3	53.0	45.7	86.2	7.3	13.8
	201	60.5	46.0	40.1	87.2	5.9	12.8
	Average	....	....	....	85.6	..	14.4
Steam distillation, solutions acidified with H_2SO_4..................	224	59.5	56.8	50.1	88.2	6.7	11.8
	226	61.7	45.8	38.5	84.1	7.3	15.9
	228	56.6	49.4	42.2	85.4	7.2	14.6
	229	57.3	34.9	28.9	82.8	6.0	17.2
	Average	....	....	....	85.1	....	14.9
Solutions acidified with H_2SO_4..................	220	59.8	37.3	32.5	87.1	4.8	12.9
	221	57.7	54.7	47.4	86.7	7.3	13.3
	273	62.0	51.3	45.5	88.7	5.8	11.3
	274	62.0	45.0	38.9	86.4	6.1	13.6
	Average	....	....	....	87.2	....	12.8
Series D, 200 grams of young leaves, fumigated 45 minutes							
Solutions acidified with H_2SO_4..................	212	76.4	38.7	31.7	81.9	7.0	18.1
	213	75.5	37.6	26.7	71.0	10.9	29.0
	214	75.9	38.6	26.1	67.6	12.5	32.4
	216	73.4	47.6	31.9	67.0	15.7	33.0
	217	75.9	39.6	32.4	81.8	7.2	18.2
	215	74.3	0.0	0.0	0.0	0.0	0.0
	Average	....	....	....	73.9	....	26.1

* All distillations were made by using direct gas or electric heat, except as indicated for the second group of tests in series C.

electric heat was applied directly to the distillation flask, except as indicated for the second group in series C. All leaves were mature except those in series D, which were about two-thirds mature. The temperature during the fumigation periods was always between 21° and 25° C, and in most cases it was 22° to 23°. The leaves were not removed from the flask before making the distillation, as had been done in the tests recorded in table 5. The distillation solution was run into the flask through the separatory funnel and none of the HCN was allowed to escape. The recoveries therefore represent not only the recoverable portion of the HCN that had been absorbed by the leaves but also that which remained unabsorbed in the flask at the end of the fumigation period.

Series A of table 8 shows that the average percentage of HCN recov-

ered from 100-gram lots of leaves fumigated for 45 minutes and distilled from water acidulated with H_2SO_4 was 89.9 per cent, and those acidulated with tartaric acid gave a recovery of 86.8 per cent.

In table 8, series B, 200-gram instead of 100-gram lots of leaves were used. The treatments were similar except that the fumigation periods were 90 instead of 45 minutes and that in the last four tests in this series the leaves were distilled in nonacidulated water. The average recoveries for the three groups in this series were: acidulated with H_2SO_4, 83.4 per cent; acidulated with tartaric acid, 75.5 per cent; and nonacidulated, 84.3 per cent.

In series C of table 8, the treatment of the first group was the same as that of the last group in series B, except that the fumigation periods were 45 minutes instead of 90. The average percentage of HCN recovered was 85.6. The second and third groups of data in series C show the comparative effects of steam distillation and distillation with direct heat. The average percentages of recovery of HCN were 85.1 and 87.2, respectively. Although no statistical studies have been made, the variations within the groups indicate that the differences are not significant.

A comparison of the amounts of HCN recovered from mature leaves and from those having reached only about two-thirds full size can be made from the results recorded in the last group of series C (mature) in table 8, and in series D (young). The average percentages of HCN recovered were 87.2 and 73.9, respectively.

THE RECOVERY OF HCN FROM CITRUS-FRUIT DISTILLATES

The green and mature citrus fruits tested were all picked from the same block of trees, a standard orange clipper being used to cut the stem. After picking they were immediately brought to the laboratory, counted into lots—usually of 15 each—weighed, and the diameters measured.

In working with the young, green orange fruits, the same precautions as had been used with the leaves were necessary. In the first tests, the green fruits were cut into small pieces and treated like the leaves. A known quantity of HCN was dissolved in a solution of NaOH of the usual strength, placed in the flask with the sliced fruits, and distilled at once.

In addition to H_2S, which had caused trouble in making determinations from the leaf extracts and which was taken care of by using $CdSO_4$, there appeared to be a substance or substances in the distillate which slowly reacted with the HCN. In order to check on this possibility, the amounts of HCN in the distillates were determined immediately after distillation and then again after the distillate had stood for 24 hours.

The results of these tests are shown in table 9. The quantities of HCN added before distillation plotted against the quantities recovered in the distillate immediately after distillation show a straight-line relation (graph not included in this paper).

TABLE 9

AMOUNTS OF HCN RECOVERED FROM THE DISTILLATE FROM GREEN AND MATURE VALENCIA FRUITS

(The HCN was added either immediately before or after distillation; there was no fumigation period)

HCN added before or after distillation	Experiment No.	Date of picking, 1937–38	Weight of fruits,* grams	HCN added, mg	HCN recovered after standing: Amount, mg, 0 hours	Amount, mg, 24 hours	Per cent, 0 hours	Per cent, 24 hours
Green Valencia fruits								
Before	287	Oct. 20	729	52.8	48.4	. . .	91.7	. . . .
Before	288	Oct. 20	724	37.2	34.4	. . . .	92.5	. . .
Before	289	Oct. 25	1,103	39.6	36.4	. . . .	91.9	. . .
Before	290	Oct. 25	1,208	48.6	44.6	. . . .	91.8	.
Before	339	Nov. 26	1,140	81.6	73.5	71.9	90.1	88.1
Before	340†	Nov. 26	1,108	27.1	25.6	24.7†	94.5	91.1†
Before	360	Dec. 17	1,144	65.5	60.7	59.6	92.7	91.0
Before	361	Dec. 17	1,242	42.3	39.4	37.7	93.1	89.1
Before	362	Dec. 20	1,341	16.7	15.2	14.3	91.0	85.6
Before	363	Dec. 20	1,300	23.9	22.5	21.6	94.1	90.4
Before	364	Dec. 21	1,464	34.6	31.7	30.1	91.6	87.0
Before	365	Dec. 21	1,390	28.5	27.2	25.6	95.4	89.8
Before	Average	. . . .	. . . . .	. . . .	. . .	. . . .	92.5	89.0
Immediately after	342‡	Nov. 29	2,248	75.0	73.1	69.6‡	97.5	92.8‡
24 hours after	343	Dec. 1	1,155	15.1	14.5	14.3	96.0	94.7
48 hours after	344	Dec. 1	1,117	39.2	37.8	37.4	96.4	95.4
Mature Valencia fruits								
Before	366	May 3	1,182	71.6	67.4	. . . .	94.1	. . . .
Before	367	May 4	1,085	34.2	31.4	30.6	91.8	89.5
Before	368	May 4	1,122	35.6	33.0	32.2	92.7	90.4
Before	369	May 5	1,184	50.0	45.8	. . . .	91.6	. . . .
Before	Average	. . . .	. . . . .	. . . .	. . . .	. . . .	92.5	90.0

* The number of fruits was 15 except as follows: experiment No. 287, 13 fruits; No. 288, 13; No. 342, 30; No. 366, 7; No. 367, 7; No. 368, 8; and No. 369, 8.

† After 72 hours, 23.7 mg, 87.5 per cent.

‡ After 48 and 72 hours, 69.0 and 68.5 mg, 92.0 and 91.3 per cent, respectively.

Further trials were made, not only by adding a known quantity of HCN immediately before distillation and by making determinations on the distillate at intervals, but also by adding known quantities of HCN to distillates from unfumigated fruits and by making determinations at once and at 24- and 48-hour intervals. The results of these tests are also

given in table 9. The leaf extracts appeared to contain similar substances, but no definite tests were made to determine the amounts of HCN that combined with them after given intervals of time.

Several months later similar tests were made on mature Valencia fruits taken from the same trees. The color of the distillate indicated that the amount of H_2S in the mature fruits was less than that in the green fruits; however, enough was present so that it was necessary to use $CdSO_4$ in the distillation flask. The amounts of HCN recovered from the different lots of mature fruits are given in the lower section of table 9.

In the next series of tests, different lots of green fruits were fumigated for 40 minutes in a 100-cu. ft. gastight metal fumatorium with different amounts of HCN.

The amounts of HCN used for fumigation at different times ranged from 0.88 mg to 3.66 mg per liter of space in the fumatorium. The fumigation temperature was always maintained at 24° C. The period of fruit aeration after fumigation ranged from 5 minutes to 44 hours; the two periods most used were 10 minutes and 22 hours. A fan kept the gases in the fumatorium in constant circulation during the entire fumigation period. The concentration of HCN in the fumatorium was measured four times during a fumigation period.

After fumigation, each fumigated fruit was cut into 10 to 15 pieces and placed in the distillation flask with 3 liters of acidulated distilled water and 30 grams of $CdSO_4$. It was necessary to make only a single distillation with the fruits because the tissues were more nearly completely immersed in the distillation liquid. In experiments 281 to 334, inclusive, the fruits were distilled by direct heating. In experiments 337 to 369, inclusive (Nos. 342 to 369 not included in table 10), they were steam-distilled. One liter of distillate was caught in NaOH as described for the leaves. After filtering the distillate, 150-ml aliquots were used for titration.

Table 10 shows the amounts of HCN recovered from green Valencia oranges that had been fumigated in the fumatorium. Four to eight lots of fruit were used in each experiment. The amount of HCN recovered was roughly in direct proportion to the concentration of HCN in the fumatorium, as shown in figure 1. The figure does not show it, but the amount of HCN recovered from the fruit was inversely proportional to the length of the aeration period. No HCN could be detected after 44 hours' aeration.

The data recorded in table 11 are similar to those in table 10, except that, although in all cases the whole fruits were fumigated, the peels and pulps of some lots were distilled separately. This was done in order to

TABLE 10

AMOUNTS OF HCN RECOVERED FROM THE DISTILLATE FROM GREEN VALENCIA ORANGES FUMIGATED WITH DIFFERENT AMOUNTS OF HCN IN THE FUMATORIUM

HCN per liter in fumatorium, mg	Date of picking fruits, 1937	Experiment No.	Length of aeration	Weight of fruits, grams	Average diameter of fruits, inches	HCN recovered, mg: Sample of 15 fruits	HCN recovered, mg: Average of 2 samples
1.10	Oct. 20	281	5 min.	1,120	2 3/32	8.8	8.3
		282	5 min.	1,201	2 1/8	7.7	
		283	22 hrs.	1,254	2 5/16	1.0	0.8
		284	22 hrs.	1,037	2	0.6	
		285	44 hrs.	1,063	2	0.0	0.0
		286	44 hrs.	1,211	2 1/8	0.0	
0.88	Nov. 17	323	15 min.	1,171	2 1/8	12.1	12.5
		324	15 min.	1,203	2 3/16	13.0	
		325*	22 hrs.	1,174	2 1/8	2.1	2.0
		326*	22 hrs.	1,182	2 1/8	1.9	
1.61	Oct. 29	299	15 min.	1,206	2 1/8	21.4	22.1
		300	15 min.	1,142	2 1/16	22.9	
1.52	Nov. 3	305	10 min.	1,111	2 1/16	27.7	29.3
		306	10 min.	1,150	2 1/16	31.0	
1.47	Nov. 9	311	10 min.	1,175	2 1/8	21.0	19.6
		312	10 min.	1,142	2 1/16	18.3	
		315	24 hrs.	1,184	2 1/8	2.6	2.6
		316	24 hrs.	1,123	2 1/16	2.6	
2.58	Oct. 26	291	5 min.	1,174	2 1/8	38.6	39.0
		292	5 min.	1,191	2 3/32	39.3	
		295	44 hrs.	1,164	2 3/32	0.0	0.0
		296	44 hrs.	1,190	2 3/32	0.0	
2.58	Oct. 27	293	22 hrs.	1,160	2 3/32	4.7	5.1
		294	22 hrs.	1,148	2 3/32	5.6	
2.46	Nov. 12	317	10 min.	1,051	2 1/16	35.2	35.6
		318	10 min.	1,106	2 1/8	36.1	
3.66	Nov. 15	319	10 min.	1,140	2 1/8	68.4	66.6
		320	10 min.	1,085	2 1/16	64.7	
3.15	Nov. 16	321	10 min.	1,120	2 1/16	58.2	59.0
		322	10 min.	1,022	2 1/16	59.8	
3.21	Nov. 19	327*	15 min.	1,126	2 1/16	57.3	54.0
		328*	15 min.	1,129	2 1/16	50.8	
		329	22 hrs.	1,143	2 1/16	8.8	9.1
		330	22 hrs.	1,170	2 1/8	9.4	
3.24	Nov. 22	331‡	15 min.	1,146	2 1/8	39.9	43.3
		332‡	15 min.	1,134	2 1/8	46.6	
		333†	22 hrs.	1,136	2 1/8	6.4	6.7
		334†	22 hrs.	1,155	2 1/8	6.9	
2.50	Nov. 26	337	15 min.	1,065	2 1/16	32.8	32.2
		338	15 min.	1,075	2 1/16	31.7	
		341	22 hrs.	1,177	2 1/8	6.6	....

*, ‡, † Tested 24, 42, and 72 hours, respectively, after distillation. No earlier titrations were made.

determine the depth of penetration of the HCN into the fruits during the fumigation period and during the interval between fumigation and distillation. In order to guard as much as possible against the loss of HCN, the fruits were dipped in distilled water before peeling and the water was added to the distillation solution. In this connection it may be

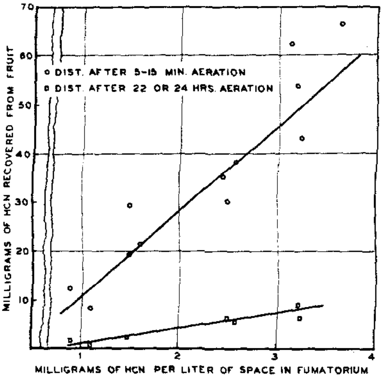

Fig. 1.—The amounts of HCN that were placed in the 100-cu.-ft. fumatorium with different lots of green Valencia fruits, and the amounts of HCN recovered from them by subsequent distillations (see table 10).

mentioned that citrus fruits are acid and such a condition favors the release of HCN. The determinations were made on all of the distillates immediately, and then again 24 or 48 hours later.

DISCUSSION

A nonvolatile organic acid of low concentration is usually recommended and used in the recovery of HCN from biological materials by the distillation method. Krieble and Peiker (*5*) found that hydrolysis of the HCN will occur, and Cobb and Walton (*4*) have shown that HCN forms complexes with H_2SO_4, if the acid concentrations are too high. However, Pagel and Carlson (*7*) and Morris and Lilly (*6*) reported satisfactory recovery when HCN was distilled from solutions acidified with H_2SO_4.

Most of the distillations reported in these experiments were made in the presence of H_2SO_4. This acid was used because, as shown in series A

and the first two groups of series B in table 8 (p. 482), a greater percentage of the HCN could be recovered by using H_2SO_4 than by using tartaric acid. Furthermore, for some unknown reason, titration to the end point was somewhat more difficult when tartaric acid was used. As has been stated already, in the earlier tests, 0.30 ml, but in the later tests only 0.15 ml, of concentrated H_2SO_4, beyond neutrality, was used in the 3 liters of solution in the distillation flask. It should be borne in mind

TABLE 11

AMOUNTS OF HCN RECOVERED FROM FUMIGATED GREEN VALENCIA ORANGES AND FROM THEIR PEELS AND PULPS SEPARATELY

(The determinations were made immediately after distillation and 24 or 48 hours later)

HCN per liter in fumatorium, mg	Date of picking, 1937	Length of aeration	Average diameter of fruits, inches	Weight of fruits, grams	Experiment No.	Part of fruit distilled	HCN recovered after standing, mg			HCN lost on standing, mg	
							0 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.
2.50	Nov. 26	*15 min.*	2 1/16	*1,065*	337	Whole	32.8	31.7	...	1.1	
		15 min.	2 1/16	1,075	338	Whole	31.7	29.7	...	2.0	
		22 hrs.	2 1/8	1,177	341	Whole	6.6	..	5.0	.	1.6
1.68	Nov. 24	15 min.	2 1/16	1,087	335	Peel	19.8	...	17.7	. .	2.1
					336	Pulp	2.6	...	2.0	.	0.6
1.59	Dec. 2	15 min.	2 1/16	1,149	345	Peel	15.8	14.4	....	1.4	...
					346	Pulp	3.2	2.3	....	0.9	...
		4 hrs.	2 1/8	1,213	347	Peel	5.5	5.0	....	0.5	.
					348	Pulp	1.9	1.2	. .	0.7	...
		22 hrs.	2 1/16	1,133	349	Peel	2.0	1.9	....	0.1	...
					350	Pulp	0.6	0.4	....	0.2	.

that neither of these concentrations of H_2SO_4 (0.0176 per cent, 0.0036 *N*, and 0.0088 per cent, 0.0018 *N*, respectively) furnished the solutions with as many free H-ions as were available in the 2 per cent (0.2666 *N*) tartaric acid solution. Table 4 (p 479) shows that the same average recovery of HCN was obtained with H_2SO_4 as with tartaric acid when the distillations were made in the absence of leaves.

Distillations were made with steam or, more often, with heat from a free flame or an electric heater. The results given in treatment C of table 2 (p. 475) show that the application of direct heat did not cause a measurable destruction of HCN, probably because of the high vapor pressure of HCN, which caused it to distill rapidly, and because of its very low concentration in the solution in the distillation flask.

The liquid HCN used in the experiments reported in this paper was

guaranteed to contain 96 per cent available HCN. Corrections have accordingly been made to a basis of 100 per cent available HCN wherever percentages of recovery are given. However, when the distillations were made in the absence of tissues, the recoveries (see table 2, treatment B, and table 4) indicate that the amount of available HCN was approximately 96.5 per cent.

All of the HCN could not be recovered from citrus leaf tissues. Repeated tests showed that no additional HCN could be recovered from the fumigated tissues by driving over more than 800 to 900 ml of distillate or by redistilling the tissues. Tests also showed that none of the HCN was escaping from the receiving vessel. This was determined by using a chain of receiving vessels. All of the HCN was caught in the first unit.

The leaves appear to contain a substance or substances which combine with a portion of the HCN in such a manner that it either is no longer volatile, or if it does distill over, will not react with standard $AgNO_3$. More HCN could be recovered when the distillations were made as soon as the HCN had been added than when the tissues were exposed to it during a 45-minute fumigation period. This is shown very well by the data in tables 6 and 8. Table 6 (p. 481) shows that 99.0 per cent of the HCN could be recovered when it was distilled at once from leaf distillate and 95.8 per cent when distilled at once in the presence of unfumigated leaves. These percentages of recovery are considerably higher than those obtained by making the distillations after a 45-minute fumigation period. Under such conditions the highest percentages of recovery from 100-gram and 200-gram samples of mature leaves were 89.9 per cent and 87.2 per cent, respectively, while the recovery from 200-gram samples of immature leaves was only 73.9 per cent (see table 8, p. 482). Such results *indicate that at least a certain amount of HCN can be chemically bound* or fixed by citrus leaf tissues. Apparently biological or other factors prevented proportionality fixation.

Because of the manner in which the fruits were fumigated, the percentages of HCN recovered from them cannot be computed. That the fruits, as well as the leaves, fixed a certain amount of the HCN during the fumigation period is indicated by the fact that when unfumigated fruits were placed in an alkaline HCN solution and distilled at once, only about 92 per cent of the HCN could be recovered (table 9, p. 485). This is more strikingly shown in results that have not been described or tabulated. When different lots of fruits were exposed to 1.61 mg of HCN per liter of space in the fumatorium and distilled, after a 15-minute period of aeration, about 22 mg of HCN was recovered. However, when other lots of fruit, which had been fumigated at the same time, were placed in

desiccators over NaOH and left for 17 and 64 hours, averages of only 8.9 mg and 2.3 mg, respectively, could be recovered from the fruits and their underlying NaOH.

In studying the cyanophoric glucosides in such plants as *Prunus virginiana* and *Andropogon Sorghum*, Alsberg and Black (*1*) found that not only all autogenous HCN in the tissues could be obtained but also that 100 per cent of any added HCN could be recovered if the distillation were made at once. These results do not agree with those reported in this paper for citrus tissues. Immediate distillation did not recover all of the added HCN, and the more finely divided the tissues were before being fumigated or the younger the tissue, the less the percentage that could be recovered. Only about 80 per cent could be recovered when the mature leaf tissues were finely ground, fumigated, and distilled at once (table 7, p. 481), and only about 74 per cent from whole, immature leaves (table 8, p. 482). From these and other studies (*10*) it appears that the amount of HCN fixed or chemically changed during and after fumigation depends upon the kind of plant tissue that is being tested.

In this connection it may be repeated that Bartholomew and Raby (*3*) found that citrus leaf and fruit tissues do not contain a cyanophoric glucoside. These results have been verified in the present investigation (for example, see experiment 211, table 7, p. 481).

Although studies in this field have been outlined, no attempt was made during the course of these experiments to determine the nature or identity of the substances which prevented the recovery of all of the HCN. Bartholomew and Raby (*3*) found that full recoveries could be made when HCN was distilled in the presence of sugars, citral, or pure citrus oils. They found, however, that small amounts of aldehydes can be recovered from green citrus tissues and that these may be at least partially responsible for the fixation of the HCN that could not be recovered after distillation in the presence of the tissues. Whether these substances or others were responsible for the progressive disappearance of titratable HCN from the distillate from citrus tissues upon standing is not known. Maximum determinations of HCN in the distillates were obtained by titrating immediately after distillation.

That the disappearance of HCN, in such dilute solutions as were used, could not have been caused except by its being combined with substances in or distilled from the tissues was determined by experimentation. Thirty-one mg of NaCN were dissolved in 1 liter of distilled H_2O, and 125 liters of air per hour were bubbled through it over a period of 24 hours. At the end of the period the solution contained the same amount of NaCN as at the beginning.

That the HCN had actually penetrated the leaves during the period of fumigation and was not merely adhering to their surfaces was shown by the fact that an average of 66.3 per cent of the HCN could be recovered from the leaves even after they had received the several washings of alkaline, distilled, and acidulated water (see table 7, p. 481). Similar results are indicated in table 5 (p. 480), where it is shown that HCN could be recovered from mature citrus leaves although they had been exposed to the laboratory air for as long as 15 hours. By the end of the 15-hour period, the leaves had become badly wilted and had lost about 27 per cent of their water content. Further evidence that the HCN penetrates into the citrus tissues is presented in table 11 (p. 489), which shows that HCN could be recovered from the pulps after the peels had been removed from fumigated whole fruits. These results are specially mentioned because of unpublished statements to the effect that HCN does not enter citrus leaves or fruits but merely adheres to their cutinized surface during the fumigation period of 40 or 45 minutes.

The first group of results given in table 5 (p. 480) were obtained by removing the leaves from the fumigation flask, exposing them to the laboratory air, and stirring them for 1 minute before distilling them to recover the HCN. The question may be raised as to whether all of the HCN had escaped from the surface of the leaves during such a short period of time. That such a time interval was sufficient is indicated by the fact that the average of the amounts of HCN recovered from leaves treated in this manner was 66.8 per cent (table 5), while the average recovered from the leaves that had received the several washings, after being fumigated and before being distilled, was 66.3 per cent (table 7, p. 481).

The results given in tables 10 and 11 (pp. 487 and 489) are expressed in milligrams rather than as percentages. Recovery percentages, calculated on the basis of the total quantity of HCN added to the fumatorium and the amount recovered from the fruit would be of little value, since a considerable quantity of the HCN was sorbed on the walls of the fumatorium; HCN is known to be strongly sorbed on surfaces of this kind. Furthermore, traces of sodium or other alkaline substances on the walls of the fumatorium would also react with the HCN. Early investigations showed that soda glass could not be used in such experiments.

As has been stated, tests were made, usually at intervals of 2, 7, 15, and 30 minutes, in order to determine the relative concentrations of HCN to which the tissues were exposed in the fumatorium during the fumigation period. The maximum decrease in the amount of HCN in the fumatorium at the time of the 30-minute test, in comparison with that present at the

time of the 2-minute test, was 14.4 per cent. The average decrease for all fumatorium tests was 8.2 per cent.

Results of unpublished experiments[7] show that this average of 8.2 per cent is very close to the average obtained when tests were made to determine the amounts of HCN that would be sorbed by the walls, pipes, and other portions of the iron fumatorium when no tissues were present. Therefore only a comparatively small proportion of the decrease in HCN in the fumatorium by the end of the fumigation period was due to absorption by the fruit that was being fumigated. However, computations from the data in table 10 (p. 487) indicate that a given volume of fruit contained from ten to fourteen times as much HCN as an equal volume of air in the fumatorium. The amounts of HCN to which the different lots of fruits were exposed, as given in tables 10 and 11 (pp. 487 and 489), are the averages of the tests made during the fumigation period and not the original amounts placed in the fumatorium.

In a preliminary report, Bartholomew and Raby (*3*) stated that a comparatively large amount of HCN was combined or fixed by substances distilled from citrus leaves in such a manner that it would not react with standard $AgNO_3$. The present investigations have shown that the amounts of HCN fixed by citrus tissues (table 6, p. 481) are not so great as they stated. The reason for the error in the preliminary work was the presence of H_2S in the distillate, which prevented the determination of the end point until excessive amounts of HCN had been added. The placing of $CdSO_4$ in the distillation flask obviated the difficulty encountered in the earlier work because it changed the volatile H_2S to nonvolatile CdS.

SUMMARY

Methods are described for the handling of HCN in amounts as small as 10 to 15 mg and for the distillation, recovery, and determination of HCN from citrus tissues.

Hydrogen sulfide from the fumigated citrus leaves and fruits passed over into the distillate and interfered with the determinations of HCN with standard $AgNO_3$. This trouble was overcome by placing $CdSO_4$ in the distillation flask at the time of making the first (fruits) or second (leaves) distillation.

Tissues of citrus leaves and fruits are already acid, so that only a small amount of concentrated H_2SO_4 had to be added to insure the recovery of the HCN during distillation. No destruction of the HCN occurred as a

[7] Lindgren, D. L. Sorption of HCN by the walls of a metal fumatorium. Unpublished material on file at University of California Citrus Experiment Station, Division of Entomology. 1935.

result of using H_2SO_4 of these concentrations, and even better recoveries of HCN were obtained than when the tissues were distilled in the presence of a 2 per cent solution (beyond neutrality) of tartaric acid.

The experimental results indicate that citrus leaves and fruits fix or alter a portion of the HCN during the fumigation period so that it cannot be recovered by distillation. Approximately 85 per cent of the HCN could be recovered from mature leaves and 73 per cent from immature leaves. Less HCN could be recovered from leaves that had been finely ground before being fumigated than from fumigated whole leaves. There was an evident fixation of a portion of the HCN. HCN penetrates into the tissues and does not merely adhere to the surface. This was shown by the tests in which the leaves were thoroughly washed before distillation and by the fact that HCN could be recovered from the pulp of fumigated fruits after they had been peeled.

Aeration tests with mature fumigated leaves showed that the amounts of HCN that could be recovered from them decreased in roughly inverse proportion to the length of time of aeration. Fifteen per cent was recovered from the leaves after 15 hours, but none could be recovered from the fruits after 44 hours.

The amounts of HCN that could be recovered from citrus leaves and fruits were directly proportional to the amounts of HCN placed in the fumatorium in these tests.

When leaf and fruit distillates which contained HCN were allowed to stand, some unknown substance in the distillate continued to combine slowly with the HCN so that it would not react when titrated with standard $AgNO_3$.

By the end of the 40-minute fumigation period in the fumatorium, a given volume of the green fruit contained from ten to fourteen times as much HCN as there was in an equal volume of air in the fumatorium.

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